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(54) Title: HUMAN GALANIN

(NH_2) Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-VAL-GLY-Asn-His-Arg-Ser-Phe-SER-Asp-Lys-ASN-Gly-Leu-THR-SER- (COOH)

(57) Abstract

Substantially pure human galanin.

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HUMAN GALANIN

Background of the Invention

This application is a continuation-in-part of Kaplan, U.S.S.N. 07/641,344, filed January 16, 1991.

Porcine galanin is a 29-amino acid, amidated neuropeptide that regulates intestinal peristalsis, as well as secretory activity of the stomach, small intestine, pituitary, hypothalmus and other parts of the central nervous system, exocrine pancreas, and pancreatic islets. Many actions of this peptide are mediated by the amino-terminal portion that is identical in porcine, bovine and rat galanins. However, differences in biological activity between porcine, rat, and human galanin suggest physiologic importance of the species-dependent carboxy-terminal region.

Summary of the Invention

The invention features substantially pure human galanin. By substantially pure is meant that the human galanin provided by the invention is about 95%, by weight, free from the proteins and other naturally occurring organic molecules with which it is naturally associated.

The invention also features any biologically active fragment or analog of human galanin. By "biologically active" is meant possessing any in vivo or in vitro activity which is characteristic of the 30-amino acid human galanin shown in Fig. 1 (SEQ ID NO:1). Because galanin exhibits a range of physiological properties and because such properties may be attributable to different portions of the galanin molecule, a useful human galanin fragment or human galanin analog is one which exhibits a biological activity in any one (or more) of a variety of galanin

assays, for example, those assays described by Ullrich and Wallheim, FEBS Lett. 247:401, 1989; Sharp et al., J. Biol. Chem. 264: 7302, 1989; Fisone et al., Proc. Natl. Acad. Sci. USA 84:7339, 1987; Mastropaolo et al. Proc. Nat. Acad. Sci. USA 85:9841, 1988; Sundstrom and Melander, Eur. J. Pharmacol. 146:327, 1988; Fox-Threlkeld, *Galanin and Gastrointestinal Function* in Galanin: A New Multifunctional Peptide in the Neuro-Endocrine System, MacMillan, London, 1991; Koenig et al., Reg. Peptides 24:81, 1989; Nordstrom et al., Neurosci. Lett. 73:21, 1987; Melander et al., Acta. Physiol. Scand. 131:25, 1987; Davis et al. J. Clin. Endocrin. and Metab. 65:1, 1987; Koshiyama et al., Brain Res. 507:321, 1990; Yau et al., Neurosci. Lett. 72:305, 1986; or Kwok et al., Eur. J. Pharmacol. 145:49, 1988. A human galanin fragment or human galanin analog possessing, most preferably 90%, more preferably 70%, preferably 40%, or at least 10% of the activity of 30-amino acid human galanin (shown in Fig. 1; SEQ ID NO:1), in any in vivo or in vitro galanin assay (e.g., those described above), is considered biologically active and useful in the invention.

Preferred human galanin fragments include amino acids 2-15 of Fig. 1 (SEQ ID NO: 1); amino acids 2-23 of Fig. 1 (SEQ ID NO:1); amino acids 15-30 of Fig. 1 (SEQ ID NO:1); amino acids 21-30 of Fig. 1 (SEQ ID NO: 1); or a combination thereof. Preferred analogs include 30-amino acid human galanin (or biologically active fragments thereof) whose sequences differ from the wild-type sequence only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not destroy the polypeptide's biological activity as measured

using in vivo or in vitro galanin assays (e.g., those described above). Preferred analogs also include human galanin (or biologically active fragments thereof) which are modified for the purpose of increasing peptide stability; such analogs may contain, for example, one or more desaturated peptide bonds or D-amino acids in the peptide sequence. Alternatively, increased stability may be conferred by cyclizing the peptide molecule.

The invention further features compounds which antagonize human galanin activity. As discussed above, galanin possesses a number of different biological activities; as such, a useful antagonist is one which decreases the activity of 30-amino acid human galanin in any in vivo or in vitro galanin assay (e.g., those described above). To test for inhibition, the candidate antagonist is added to the assay reaction mixture or test organism either before, along with, or less preferably after addition of 30-amino acid human galanin. Galanin activity is measured and compared with a control assay in which only 30-amino acid galanin is added or administered. Any compound which decreases galanin activity (relative to the wild-type human galanin control) is considered to be a useful antagonist within the scope of the invention. Most preferably, antagonists decrease 30-amino acid human galanin activity by at least 70%; more preferably, antagonists decrease 30-amino acid human galanin activity by at least 50%; and preferably, antagonists decrease 30-amino acid human galanin activity by at least 10% in the appropriate in vivo or in vitro galanin assay.

Preferred antagonists include inhibitory fragments or analogs of the human galanin protein itself. Any human galanin fragment or human galanin analog which decreases galanin activity (relative to the wild-type human galanin control) is considered to be a polypeptide

within the scope of the invention. Inhibitory human galanin fragments or analogs may be engineered to increase their stability in vivo, for example, by addition of D-amino acids or unsaturated peptide bonds, or by cyclization of the molecule (as described above).

The human galanin of the invention or any fragment or analog thereof can be prepared either by conventional solid phase peptide synthesis, or by culturing of recombinant cells containing DNA sequences (e.g., purified DNA sequences) encoding the human galanin polypeptide, and isolating the human galanin (or fragment or analog) therefrom.

Purified DNAs encoding human galanin, biologically active fragments or analogs of human galanin, or inhibitory (antagonist) fragments or analogs of human galanin are also featured in the invention. By "purified DNA" is meant a DNA molecule which encodes a human galanin polypeptide (or an appropriate fragment or analog), but which is free of the genes that, in the human genome, flank the galanin gene. An example of purified human galanin DNA (i.e., human galanin cDNA) is shown in Fig. 3. The invention features DNA of this sequence as well as DNA of substantially identical sequence. By "substantially identical" is meant a nucleic acid sequence encoding an amino acid sequence which differs only by conservative amino acid substitutitions, for example, substitution of one amino acid for another of the same class or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the biological activity of the human galanin polypeptide (as determined using any in vivo or in vitro assay, for example, those described above).

The human galanin of the invention possesses a number of physiological properties which give it potential as a therapeutic agent having several significant applications. The first such application is in birth control; a number of experimental results, described in greater detail below, indicate that fertility can be decreased by administering to a woman human galanin (or a biologically active fragment or analog) in an amount sufficient to inhibit release of one or more hormones necessary for reproduction. Galanin can be expected to exhibit a number of advantages over prior art birth control methods such as the use of estrogen-containing formulations, which can cause serious side effects such as increased risk of mammary carcinoma. Galanin, in contrast, should avoid those serious side effects, as it may represent a birth control mechanism devised by evolution, and may in fact be the hormone which naturally prevents pregnancy in lactating women. Furthermore, the human female reproductive system can be expected to return to normal shortly after discontinuing galanin administration. Similarly, administration of a galanin antagonist (e.g., an inhibitory galanin fragment or analog) can be expected to stimulate ovulation and act as a fertility agent.

A second potential therapeutic use of galanin (or a biologically active fragment or analog thereof) is in the management of pain. A recently-published paper by other workers reports that fragments of rat galanin were found to augment the analgesic effect of morphine in humans. The human peptide can be expected to exhibit analgesic effects as well, and can be administered according to the invention alone or in combination with other analgesic agents such as morphine.

An additional therapeutic use of human galanin (or a biologically active fragment or analog thereof) is in

the treatment of irritable bowel syndrome. Conversely, a human galanin antagonist (e.g., an inhibitory human galanin fragment or analog) may act as a pro-motility agent, useful for the treatment of constipation ileus, gastroparesis diabeticorum, or chronic idiopathic pseudoobstruction. For these uses, the galanin polypeptide (or galanin antagonist) may be formulated so that it is protected from the gastric acid in the patient's stomach for a period of time sufficient to allow the composition to pass undisintegrated into the patient's small intestine; this can be achieved by conventional coating and encapsulation techniques.

Another therapeutic use of human galanin (or a biologically active fragment or analog thereof) is in the treatment of anorexia, which can be caused by cancer, chemotherapy used to treat cancer, and other neurologic diseases which cause a decrease in appetite. Conversely, human galanin antagonists (e.g., inhibitory human galanin fragments or analogs) may be used to treat obesity. Because of their complementary effects, human galanin (or a biologically active human galanin fragment or analog) and human galanin antagonists (e.g., inhibitory galanin fragments or analogs) may be administered, alone or in the appropriate combination, to selectively alter an individual's food preferences between carbohydrates, proteins, and fats, thereby encouraging an individual to maintain an ideal diet.

A further therapeutic use of human galanin (or a biologically active fragment or analog thereof) is in the treatment of insulin hypersecretory states, caused by insulinoma, nesidioblastosis, and other hypoglycemic syndromes. Human galanin antagonists (e.g., inhibitory human galanin fragments and analogs) are useful in the treatment of insulin hyposecretory syndromes, such as diabetes.

A final therapeutic use of human galanin (or a biologically active fragment or analog thereof) is in the treatment of growth hormone deficiencies leading, for example, to short stature. Galanin stimulates growth hormone secretion, suggesting that its administration may trigger the release of human growth hormone in a patient and thereby promote increased size.

Accordingly, to make therapeutic compositions, human galanin (or any biologically active human galanin fragment or human galanin analog or any human galanin antagonsist, e.g., any inhibitory human galanin fragment or analog) is admixed with a therapeutically effective amount of a pharmaceutically acceptable carrier substance (e.g. magnesium circinate, lactose, or a phospholipid with which the therapeutic compound can form a micelle). Such compositions can be in the form of a pill, tablet, capsule, or liquid for oral administration to a human patient, a liquid capable of being administered nasally as drops or spray, or a liquid capable of intravenous, parenteral, intrathecal, subcutaneous, or intraperitoneal administration. Intrathecal administration may be particularly important where the blood-brain barrier is a consideration, as may be expected to be the case in the treatment of pain and the improvement of appetite. The therapeutic composition can also be administered in the form of an oil emulsion or dispersion in conjunction with a lipophilic salt such as a pyemic acid. The therapeutic composition can also be in the form of a sustained release formulation for intramuscular administration. Release can also be achieved using an implantable or external pump, e.g., an Infusaid™ pump. Dosage will normally be in the range of 0.01 to 50 mg/kg/day, preferably 0.1 to 5 mg/kg/day.

Also featured in the invention is the use of human galanin (or a biologically active human galanin fragment

or human galanin analog) in the manufacture of a medicament for decreasing fertility in a human female patient, decreasing pain, treating irritable bowel syndrome, treating anorexia, or treating an insulin hypersecretory state; and the use of a human galanin antagonist (e.g., an inhibitory human galanin fragment or human galanin analog) for increasing fertility in a human female patient, increasing intestinal motility (e.g., to treat constipation ileus, gastroparesis diabeticorum, or chronic pseudoobstruction), or treating diabetes.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first described.

Drawings

Fig. 1 (SEQ ID NO:1) is the predicted amino acid sequence of human galanin. Variations from rat galanin are underlined, and variations from porcine galanin are indicated by capital letters.

Fig. 2 is a schematic diagram of preprogalanin mRNA's and peptides from rat, porcine, bovine, and human sources.

Fig. 3 (SEQ ID NO: 2) is the nucleic acid sequence of the full-length human galanin cDNA.

Cloning of the Gene for Human Galanin

A cDNA encoding human galanin was isolated from a cDNA library prepared from hypothalamic tissue. The library was screened at low stringency with mixed oligonucleotide probes corresponding to the amino-terminus of rat galanin, generally as described in Kaplan et al. (1988) Proc. Natl. Acad. Sci. USA 85:1065-1069. Sequence analysis of isolated clones revealed that human galanin is encoded as part of a 123-amino acid precursor

peptide that also includes a signal sequence and a 59-amino acid extension peptide (Figs. 2 and 3). Although the amino-terminal 15 amino acids of human galanin are identical to the rat, pig, and cow peptides, the structure of the carboxy-terminal region reveals human galanin to be the most divergent of the four known species. Genomic DNA blot hybridization analysis and chromosomal localization were consistent with a single human galanin gene.

The amino-terminal signal sequence likely mediates transfer of the nascent peptide into the endoplasmic reticulum. Within the precursor, the galanin sequence is flanked by pairs of basic amino acids, suggesting that the mature peptide is first cleaved from the precursor by trypsin-like endoproteases. Rat and human preprogalanin also include an approximately 60-amino acid extension peptide. As shown in Figure 2, this peptide (galanin mRNA-associated peptide; GMAP) contains a region that has been highly conserved among the four known galanin cDNA's (Rokaeus and Brownstein, Proc. Natl. Acad. Sci. USA 83:6287, 1986; Vrontakis et al., J. Biol. Chem. 35:16755, 1987; Kaplan et al., 1988, supra; Rokaeus and Carlquist, FEBS Lett. 234:400, 1988). This degree of sequence homology suggests that the galanin gene may encode an additional bioactive peptide. cDNA sequences predict that each rat, porcine, and bovine galanin is a 29-amino acid peptide amidated at the carboxy terminus; a glycine residue in the precursor serves as an amide donor. In contrast, cDNA's encoding human preprogalanin predict that human galanin is a 30-amino acid, non-amidated peptide.

Regulation of Human Galanin Gene Expression

Northern blot analysis with a human galanin cDNA probe was used to examine the distribution of galanin gene expression in human tissues. In contrast to the

pattern of mRNA distribution in rat, highest human mRNA levels were detected in the pituitary, with considerably lower expression in the hypothalamus and gastrointestinal tract. Galanin mRNA concentrations in the human pituitary were similar in men and women, suggesting that circulating estrogens have little effect on human galanin gene expression.

Pituitary Cell Type Distribution

Immunocytochemistry, immunoelectron microscopy, and in situ hybridization analysis were used to examine the cellular localization of galanin mRNA and peptide in human pituitary. In contrast to the rat, in humans galanin immunoreactivity was present in a subset of corticotrophs scattered throughout the gland, but not in lactotrophs, somatotrophs, or gonadotrophs. Galanin mRNA was also located predominantly in ACTH-containing cells. Coexistence of galanin and ACTH immunoreactivity was observed in hyperplastic corticotrophs and Crook's hyalinized cells in patients with Cushing's disease, as well as in basophil invasion cells of the posterior pituitary. In parallel with the studies of normal human pituitary, galanin immunoreactivity was examined in 62 pituitary adenomas (Table 1). Eighty-four percent of corticotrophic cell tumors, 14% of prolactinomas, 45% of somatotrophic cell tumors, and 50% of non-functioning adenomas contained immunoreactive galanin. Of note, however, both of the prolactinomas, 4 of the 5 somatotrophic cell adenomas, and 2/3 of the non-functioning tumors that expressed galanin also expressed ACTH, underscoring the close correlation between expression of these two peptides.

Table 1. Corr lation of Galanin- and ACTH-Immunoreactivity in Human Pituitary Tumors

Tumor Type	N Gal-IR(+)	% Gal-IR(+) that are also ACTH-IR (+)
Corticotrophic	19	84	100
Somatotrophic	11	45	83
Prolactinoma	14	14	100
Nonfunctioning	18	50	67

Regulation of Galanin Gene Expression in PC12 Cells

PC12 cells appear to provide an excellent model of regulated galanin gene expression. This cell line, derived from a malignant tumor of adrenal medullary cells, responds to nerve growth factor (NGF) by extending neurites and expressing several neuron-specific genes. In the absence of NGF, these cells assume a chromaffin cell phenotype and contain little or no galanin mRNA. However, NGF treatment induces high levels of galanin gene expression in a dose- and time-dependent fashion. Treatment of PC12 cells with glucocorticoids, which appears to reinforce the chromaffin phenotype, also increases galanin gene expression. These observations suggest that the two differentiation states of PC12 cells mimic the situation observed *in vivo*: galanin expression in endocrine cells such as pituitary lactotrophs is strongly dependent on hormonal stimulation, while expression of this gene is observed in neurons in the absence of specific external stimuli. The wide variations in mRNA and peptide levels in the pituitary suggest that galanin activity may provide a "fine tuning" mechanism for other pituitary processes. Analogous to the fine tuning required for sensitive optical and electronic equipment, large amplitude variations in galanin expression may be required to generate modest physiologic effects. In this way, small changes in

galanin levels would "micromanage" these systems. The observation that variations in galanin peptide concentrations are frequently associated with large changes in mRNA levels suggests a dynamic state in which galanin is rapidly synthesized in response to specific physiologic demands. Conversely, galanin may be rapidly degraded after those demands are met. This model is significantly different from the pattern of regulation for many other hormones and neurotransmitters, whose intracellular concentrations vary little despite large changes in secretion. Under conditions of low circulating estrogens, pituitary galanin peptide concentrations are low, indicating a considerably smaller pool size than for other anterior pituitary hormones. Therefore, galanin may not act as a classic hormone within the anterior pituitary, but that it may act *internally* to modulate cell function. Preliminary support for such a model comes from observations that galanin in rat pituitary lactotrophs is more prevalent in the Golgi apparatus than in secretory granules (Hsu et al., (1990) Endocrinology, 26, 3159-3167). The cell type distribution of galanin within the rat pituitary is also consistent with this idea.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kaplan, Lee Michael

(ii) TITLE OF INVENTION: HUMAN GALANIN

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY:	Boston
(D) STATE:	Massachusetts
(E) COUNTRY:	U.S.A.
(F) ZIP:	02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:	3.5" Diskette, 1.44 Mb
(B) COMPUTER:	IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM:	IBM P.C. DOS (Version 3.30)
(D) SOFTWARE:	WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:	
(B) FILING DATE:	
(C) CLASSIFICATION:	

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:	07/641,344
(B) FILING DATE:	16-JAN-1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:	Clark, Paul T.
(B) REGISTRATION NUMBER:	30,162
(C) REFERENCE/DOCKET NUMBER:	00786/075002

(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX:	(617) 542-8906
(C) TELEX:	200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) **LENGTH:** 30
(B) **TYPE:** amino acid
(D) **TOPOLOGY:** linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Pro His Ala
5 10 15

Val Gly Asn His Arg Ser Phe Ser Asp Lys Asn Gly Leu Thr Ser
20 25 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) **LENGTH:** 740
(B) **TYPE:** nucleic acid
(C) **STRANDEDNESS:** single
(D) **TOPOLOGY:** linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCGGACACGT GGAGGGACCC GGCCCGCGCC TTCTGCCCCT GCTGCCGGCC GCGCCATGCG 60
TGAGCGCCCC AGGCCGCCAG AGCCCACCCG ACCCGGCCCG ACGCCTGGAC CTGCCGCCCA 120
GACCCGCCAC CGCACCCGGA CCCCGACGCT CCGAACCCGG GCGCACGGCA GCTCAAGATG 180
GCCCGAGGCA GCGCCCTCCT GCTCGCCTCC CTCCTCCTCG CCGCGGCCCT TTCTGCCTCT 240
CCGGGGCTCT GGTCGCCGGC CAAGGAAAAA CGAGGCTGGA CCCTGAACAG CGCGGGCTAC 300
CTGCTGGGCC CACATGCCGT TGGCAACCAC AGGTCATTCA GCGACAAGAA TGGCCTCACC 360
AGCAAGCGGG AGCTGCGGCC CGAAGATGAC ATGAAACCAG GAAGCTTTGA CAGGTCCATA 420
CCTGAAAACA ATATCATGCG CACAATCATT GAGTTTCTGT CTTTCTTGCA TCTCAAAGAG 480
GCCGGTGCCC TCGACCGCCT CCTGGATCTC CCCGCCGCAG CCTCCTCAGA AGACATCGAG 540
GGTCCTGAG AGCCTCCTGG GCACGTTTGT CTGTGTGCTG TAACCTGAAG TCAAACCTTA 600
AGATAATGGA TAATCTTCGG CCAATTTATG CAGAGTCAGC CATTCCTGTT CTCTTTGCCT 660
TGATGTTGTG TTGTTATCAT TTAAGATTTT TTTTTTTTGG TAATTATTTT GAGTGGCAAA 720
ATAAAGAATA GCAATTAAAA 740

Claims

1. Substantially pure human galanin.

2. The substantially pure human galanin of claim 1, comprising the amino acid sequence Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Try-Leu-Gly-Pro-His-Ala-Val-Gly-Asn-His-Arg-Ser-Phe-Ser-Asp-Lys-Asn-Gly-Leu-Thr-Ser (SEQ ID NO: 1).

3. A polypeptide comprising a biologically active fragment or analog of human galanin.

4. The polypeptide of claim 3, comprising
(a) amino acids 2-15 of Fig. 1 (SEQ ID NO:1);
(b) amino acids 2-23 of Fig. 1 (SEQ ID NO:1);
(c) amino acids 15-30 of Fig. 1 (SEQ ID NO:1);
(d) amino acids 21-30 of Fig. 1 (SEQ ID NO:1); or
(e) a combination thereof.

5. A polypeptide comprising a galanin fragment or galanin analog which inhibits a biological activity of human galanin.

6. Purified DNA which encodes a polypeptide of claims 1, 2, 3, or 5.

7. The purified DNA of claim 6, said DNA comprising a nucleic acid sequence substantially identical to the nucleic acid sequence of Fig. 3 (SEQ ID NO: 2).

8. A recombinant cell containing a DNA sequence encoding (a) human galanin; (b) a biologically active human galanin fragment or human galanin analog; or (c) an inhibitory human galanin fragment or human galanin analog.

9. A therapeutic composition comprising (a) human galanin; (b) a biologically active human galanin fragment or human galanin analog; or (c) an inhibitory human galanin fragment or human galanin analog admixed with a pharmaceutically acceptable carrier substance.

10. Use of human galanin or a biologically active fragment or analog thereof in the manufacture of a medicament for

(a) decreasing fertility in a female human patient;

(b) decreasing pain in a human patient;

(c) treating irritable bowel syndrome in a human patient;

(d) treating anorexia in a human patient;

(e) treating an insulin hypersecretory state in a human patient; or

(f) treating a growth hormone deficiency in a human patient.

11. Use of a human galanin antagonist in the manufacture of a medicament for

(a) increasing fertility in a female human patient;

(b) increasing intestinal motility;

(c) treating constipation ileus;

(d) treating gastroparesis diabeticorum;

(e) treating chronic pseudoobstruction;

(f) treating obesity in a human patient; or

(e) treating diabetes in a human patient.

12. The use of claim 11, wherein said human galanin antagonist is an inhibitory human galanin fragment or inhibitory human galanin analog.

SEQUENCE OF HUMAN GALATIN

(NH_2) Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-VAL-GLY-Asn-His-Arg-Ser-Phe-SER-Asp-Lys-ASN-Gly-Leu-THR-SER- (COOH)

FIG. 1

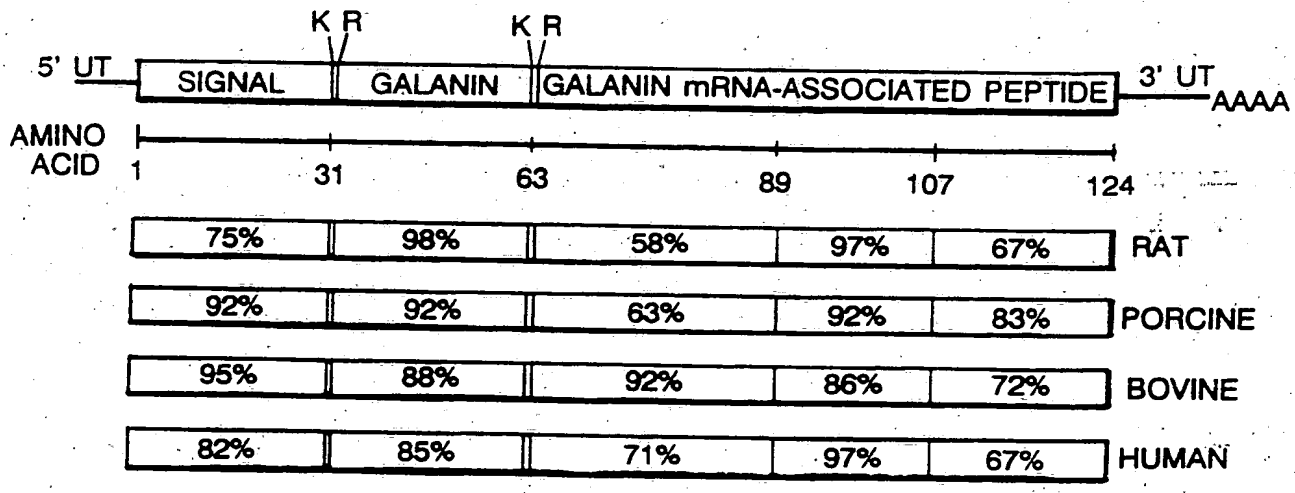

FIG. 2

```
1
CCGGACACGT  GGAGGGACCC  GGCCCGCGCC  TTCTGCCCCT  GCTGCCGGCC  GCGCCATGCG
TGAGCGCCCC  AGGCCGCCAG  AGCCCACCCG  ACCCGGCCCG  ACGCCTGGAC  CTGCCGCCCA
GACCCGCCAC  CGCACCCGGA  CCCCGACGCT  CCGAACCCGG  GCGCACGGCA  GCTCAAGATG
GCCCGAGGCA  GCGCCCTCCT  GCTCGCCTCC  CTCCTCCTCG  CCGCGGCCCT  TTCTGCCTCT
GCGGGGCTCT  GGTCGCCGGC  CAAGGAAAAA  CGAGGCTGGA  CCCTGAACAG  CGCGGGCTAC
CTGCTGGGCC  CACATGCCGT  TGGCAACCAC  AGGTCATTCA  GCGACAAGAA  TGGCCTCACC
AGCAAGCGGG  AGCTGCGGCC  CGAAGATGAC  ATGAAACCAG  GAAGCTTTGA  CAGGTCCATA
CCTGAAAACA  ATATCATGCG  CACAATCATT  GAGTTTCTGT  CTTTCTTGCA  TCTCAAAGAG
GCCGGTGCCC  TCGACCGCCT  CCTGGATCTC  CCCGCCGCAG  CCTCCTCAGA  AGACATCGAG
CGGTCCTGAG  AGCCTCCTGG  GCACGTTTGT  CTGTGTGCTG  TAACCTGAAG  TCAAACCTTA
AGATAATGGA  TAATCTTCGG  CCAATTTATG  CAGAGTCAGC  CATTCCTGTT  CTCTTTGCCT
TGATGTTGTG  TTGTTATCAT  TTAAGATTTT  TTTTTTTTGG  TAATTATTTT  GAGTGGCAAA
ATAAAGAATA  GCAATTAAAA
                      740
```

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00368

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C 07K 7/04; A 61K 37/02
US CL : 530/324, 300; 514/2

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	530/324, 300, 325, 326, 327, 328, 329, 330; 514/2; 536/27, 935/11, 13

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

Please See Attached Sheet.

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	PEDIATRIC RESEARCH, Volume 26, No. 4, issued 1989, Loche et al., "The Effects of Galanin on Growth Hormone Secretion in Children of Normal and Short Stature", pages 316-319, see entire document.	9-10
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Volume 83, issued September 1986, Rökaeus et al., "Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor", pages 6287-6291, see entire document.	1-5, 9
X/Y	GASTROENTEROLOGY, Volume 91, No. 4, issued October 1986, Bauer et al., "Distribution and Molecular Heterogeneity of Galanin in Human, Pig, Guinea Pig, and Rat Gastrointestinal Tracts", pages 877-883, see entire document.	3-5,9/1-2
Y	FEBS LETTERS, Volume 234, No. 2, issued July 1988, Rökaeus et al., "Nucleotide sequence analysis of cDNAs encoding a bovine galanin precursor protein in the adrenal medulla and chemical isolation of bovine gut galanin", pages 400-406, see entire document.	1-5,9

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
30 MARCH 1992	20 APR 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Gabriele E. Bugaisky

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X/Y	FEBS LETTERS, Volume 164, No. 1, issued November 1983, Tatemoto et al., "Galanin-a novel biologically active peptide from porcine intestine", pages 124-128, see entire document.	3-5,9/1,2,10

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

Please See Attached Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-5,9-10 (Telephone Practice)

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.